CONFIRMATION

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

REC'D 09 JAN 2006
WIPO PCT

Applicant's or agent's file reference FP2484/CKM	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/SG2004/000416	International filing date *(day/month/year)* 17 December 2004	Priority date *(day/month/year)* 17 December 2003

International Patent Classification (IPC) or national classification and IPC

Int. Cl.

C12Q 1/68 (2006.01) *C12N 15/50* (2006.01)

Applicant

AGENCY FOR SCIENCE,TECHNOLOGY AND RESEARCH et al

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [X] *(sent to the applicant and to the International Bureau)* a total of 6 sheets, as follows:
      - [X] sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      - [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - [X] Box No. I Basis of the report
   - [ ] Box No. II Priority
   - [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - [ ] Box No. IV Lack of unity of invention
   - [X] Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - [X] Box No. VI Certain documents cited
   - [ ] Box No. VII Certain defects in the international application
   - [ ] Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
7 October 2005	21 December 2005
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **LEXIE PRESS** Telephone No. (02) 6283 2677

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SG2004/000416

Box No. I Basis of the report

1. With regard to the **language**, this report is based on:

[X] The international application in the language in which it was filed

[ ] A translation of the international application into , which is the language of a translation furnished for the purposes of:

[ ] international search (under Rules 12.3(a) and 23.1 (b))

[ ] publication of the international application (under Rule 12.4(a))

[ ] international preliminary examination (Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

[ ] the international application as originally filed/furnished

[X] the description:

pages **1-31** as originally filed/furnished
pages* received by this Authority on . with the letter of
pages* received by this Authority on with the letter of

[X] the claims:

pages as originally filed/furnished
pages* as amended (together with any statement) under Article 19
pages* **32-37** received by this Authority on **7 October 2005** with the letter of **6 October 2005**
pages* received by this Authority on with the letter of

[X] the drawings:

pages **1/5-5/5** as originally filed/furnished
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

[X] a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages
[ ] the claims, Nos.
[ ] the drawings, sheets/figs
[ ] the sequence listing *(specify)*:
[ ] any table(s) related to the sequence listing *(specify)*:

4. [ ] This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

[ ] the description, pages
[ ] the claims, Nos.
[ ] the drawings, sheets/figs
[ ] the sequence listing *(specify)*:
[ ] any table(s) related to the sequence listing *(specify)*:

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims **1-22**	**YES**
	Claims	**NO**
Inventive step (IS)	Claims **1-22**	**YES**
	Claims	**NO**
Industrial applicability (IA)	Claims **1-22**	**YES**
	Claims	**NO**

2. Citations and explanations (Rule 70.7)

The present invention relates to primers for the amplification of segments from the nsp1 region of the SARS coronavirus and the use of the primers in PCR based diagnostic tests for SARS-CoV.

The following documents cited in the International Search Report have been considered for the basis of this report:

D1 Ruan et al (2003) The Lancet. Vol 361(9371): 1779-1785

D2 WO 2004085455 A1

D3 WO 2004099440 A1

D4 WO 2005005658 A1

D5 Snijder et al. (2003) Journal of Molecular Biology. Vol 331: 991-1004

D6 Yam et al. (2003) Journal of Clinical Microbiology. Vol 41(10): 4521-4524

D7 Poon et al (2003) Clinical Chemistry. Vol 49(6): 953-95

Novelty and Inventive Step

D1 is the only category X citation cited in the search report. D1 teaches the full-length genomic sequence of 14 SARS-CoV isolates and compares SARS-CoV sequences with the sequence of coronaviridae from other species. The citation indicates the position and frequency of nucleotide variations, including variations in the nsp1 region, in the 14 isolates.

D1 does not teach or suggest improved SARS-CoV, PCR based diagnostics selective for the region of the SARS coronavirus genome from nucleotide 4609 to nucleotide 4765 or from nucleotide 6652 to nucleotide 7003, or the specific amplification primers set forth in SEQ ID NOS: 3, 4, 6, 7, 9 and 10.

Therefore the subject matter of claims 1-22 meets the criteria set forth in PCT Article 33(2) for novelty and PCT Article 33(3) for inventive step.

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date (valid claim) *(day/month/year)*
WO 2004085455	7 October 2004	24 March 2004	23 March 2003
WO 2004099440	18 November 2004	9 May 2003	9 May 2003
WO 2005005658	20 January 2005	14 July 2003	14 July 2003

Each document was published after the priority date of the present application, however their priority dates precede the priority of the present application. As such each citation may be relevant at national phase examination as a whole of contents citation.

WO 2004085455 discloses the entire genomic DNA sequence of the SARS virus strain HKU-39849 (SEQ ID NO: 15) and a method for detecting the virus in a sample by RT-PCR using primers derived from a nucleotide sequence of the SARS virus. Consequently, WO 2004085455 may be relevant to the novelty of the claims at national phase examination.

(continued in Supplemental Box)

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*

Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material
      - [X] a sequence listing
      - [ ] table(s) related to the sequence listing

   b. format of material
      - [ ] on paper
      - [X] in electronic form

   c. time of filing/furnishing
      - [ ] contained in the international application as filed
      - [X] filed together with the international application in electronic form
      - [ ] furnished subsequently to this Authority for the purposes of search and/or examination
      - [ ] received by this Authority as an amendment* on

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: V1 (Certain Documents Cited) .

WO 2004099440 discloses methods, including RT-PCR, for detecting the presence of SARS in a sample. Primers for use in the method are exemplified in Table 4. Many of the primers, such as PMSU_00002 are derived from the *nsp1* gene. As such, WO 2004099440 may be prejudicial to the novelty of the present claims during national phase examination.

WO 2005005658 teaches similar methods and primers to those disclosed in WO 2004099440. Primers for the amplification of the SARS virus are exemplified in Table 18 and include several primers that span the *nsp1* gene. WO 2005005658 may be prejudicial to the novelty of the present claims during national phase examination.

Claims

1. A method for detecting SARS coronavirus nucleic acid in a sample comprising:
A) amplifying a nucleic acid of said sample with a reverse transcriptase and at least one primer specific for a NSP1 region of a SARS coronavirus to generate a nucleic acid amplification product, wherein the at least one primer specific for the NSP1 region of the SARS coronavirus is a primer consisting essentially of a sequence selected from the group consisting of SEQ ID NOs: 3, 4, 6, 7, 9 and 10; and
B) analyzing said amplification product wherein detecting an expected nucleic acid amplification product indicates the presence of SARS coronavirus nucleic acid in the sample.

2. The method of claim 1, wherein the at least one primer specific for the NSP1 region of the SARS coronavirus is a primer consisting of a sequence selected from the group consisting of SEQ ID NOs: 3, 4, 6, 7, 9 and 10.

3. The method of claim 1, wherein a primer having the nucleotide sequence of SEQ ID NO: 3 and a primer having the nucleotide sequence of SEQ ID NO: 4 are used and the expected nucleic acid amplification product is detected as a polynucleotide that is 352 nucleotides long.

4. The method of claim 1, wherein a primer having the nucleotide sequence of SEQ ID NO: 6 and a primer having the nucleotide sequence of SEQ ID NO: 7 are used and the

expected nucleic acid amplification product is detected as a polynucleotide that is 157 nucleotides long.

5. The method of claim 1, wherein a primer having the nucleotide sequence of SEQ ID NO: 9 and a primer having the nucleotide sequence of SEQ ID NO: 10 are used and the expected nucleic acid amplification product is detected as a polynucleotide that is 77 nucleotides long.

6. The method of any one of claims 1 to 5, wherein said nucleic acid amplification product is analyzed by chromatography.

7. The method of claim 6 wherein the nucleic acid amplification is performed by a polymerase chain reaction.

8. A method for detecting SARS coronavirus nucleic acid in a sample comprising:
A) amplifying a nucleic acid of said sample with a reverse transcriptase and a first primer having the sequence of SEQ ID NO: 9 and a second primer having the sequence of SEQ ID NO: 10 to generate a nucleic acid amplification product;
B) detecting said nucleic acid amplification product by specific hybridization of a probe having the sequence of SEQ ID NO: 11;
wherein detection of a specifically hybridizing amplified nucleic acid fragment indicates the presence of SARS coronavirus nucleic acid in the sample.

9. The method of claim 8, in which the nucleic acid amplification is performed by a polymerase chain reaction.

10. A method for detecting the presence of SARS coronavirus nucleic acid in a sample, comprising:

i) amplifying nucleic acids present in the sample using a forward primer and a reverse primer selective for the region of the SARS coronavirus genome from nucleotide 6652 to nucleotide 7003, said primers having a certain primer length in nucleotides and being separated by a separation length that is a certain number of nucleotides, to obtain an amplification product;

ii) determining the length of the amplification product in nucleotides;

iii) the presence of an amplification product having a length in nucleotides that is the sum of the forward primer length, the reverse primer length and the separation length indicating the presence of SARS coronavirus nucleic acid in the sample.

11. The method of claim 10, in which the nucleic acid amplification is performed by a polymerase chain reaction.

12. A method for detecting the presence of SARS coronavirus nucleic acid in a sample, comprising:

i) amplifying nucleic acids present in the sample using a forward primer and a reverse primer selective for the region of the SARS coronavirus genome from nucleotide 6652 to nucleotide 7003 to obtain an amplification product; and

ii) detecting the amplification product by specific hybridization of a probe having a nucleotide sequence of at least 18 contiguous nucleotides of the portion of the SARS coronavirus genome from nucleotide 6652 to 7003;

specific hybridization of the probe to the amplification product indicating the presence of SARS nucleic acid in the sample.

13. The method of claim 12, in which the nucleic acid amplification is performed by a polymerase chain reaction.

14. A method for detecting the presence of SARS coronavirus nucleic acid in a sample, comprising:

i) amplifying nucleic acids present in the sample using a forward primer and a reverse primer selective for the region of the SARS coronavirus genome from nucleotide 4609 to nucleotide 4765, said primers having a certain primer length in nucleotides and being separated by separation length that is a certain number of nucleotides, to obtain an amplification product;

ii) determining the length of the amplification product in nucleotides;

iii) the presence of an amplification product having a length in nucleotides that is the sum of the forward primer length, the reverse primer length and the separation length indicating the presence of SARS coronavirus nucleic acid in the sample.

15. The method of claim 14, in which the nucleic acid amplification is performed by a polymerase chain reaction.

16. A method for detecting the presence of SARS coronavirus nucleic acid in a sample, comprising:

i) amplifying nucleic acids present in the sample using a forward primer and a reverse primer selective for the region of the SARS coronavirus genome from nucleotide

4609 to nucleotide 4765 to obtain an amplification product; and

ii) detecting the amplification product by specific hybridization of a probe having a nucleotide sequence of at least 18 contiguous nucleotides of the portion of the SARS coronavirus genome from nucleotide 4609 to 4765;
specific hybridization of the probe to the amplification product indicating the presence of SARS coronavirus nucleic acid in the sample.

17. The method of claim 16, in which the nucleic acid amplification is performed by a polymerase chain reaction.

18. A SARS coronavirus detection kit comprising at least one primer for a nucleic acid amplification reaction selected from the group consisting of SEQ ID NOs: 3, 4, 6, 7, 9 and 10.

19. The SARS coronavirus detection kit according to claim 18, that comprises at least one pair of primers selected from the group consisting of a primer having the sequence of SEQ ID NO: 3 and a primer having the sequence of SEQ ID NO: 4, a primer having the sequence of SEQ ID NO: 6 and a primer having the sequence of SEQ ID NO: 7, and a primer having the sequence of SEQ ID NO: 9 and a primer having the sequence of SEQ ID NO: 10.

20. The SARS coronavirus detection kit according to claim 18 or 19, that also comprises a SARS coronavirus genomic nucleic acid, or at least a portion thereof comprising the NSP1 region.

21. The SARS coronavirus detection kit according to claim 20, in which the SARS coronavirus genomic nucleic acid has the nucleotide sequence of SEQ ID NO: 1, or the RNA equivalent thereof.

22. Use of an oligonucleotide having a sequence selected from the group consisting of SEQ ID NOs: 3, 4, 6, 7, 9, 10 and 11, in a method for detecting the presence of SARS coronavirus nucleic acids in a sample.